CYPRESS DIAGNOSTICS

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96 Test

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effegiemme diagnostici

Amebiasis Ab
E. histolytica IgG

Amebiasis E. histolytica IgG test is an enzyme immunoassay for the detection of specific IgG antibodies against Anti - Entamoeba histolytica in human serum.

To perform the test following the recommended procedure requires approximately 50 minutes.

General Information

Entamoeba histolytica is a protozoan parasite which is the causative agent of the disease amebiasis. This organism is endemic throughout the world in developing countries, but is fairly rare in most industrialized nations. When tissues are invaded by this parasite, antibodies are often formed that can be useful in detecting the infection.

Serological tests are useful I both detecting infection by E. histolytica and in excluding the organism from the diagnosis of other disorders (e.g. chronic liver diseases, ulcerative colitis, etc.).

A positive serological result however may not necessarily indicate an active infection since antibodies may persist for years after clinical cure. Also, the titer of the antibody response is not directly correlated with the severity of the infection.

A negative serological result however can be equally important in excluding suspected tissue invasion by E. histolytica.

The antibody response will vary due to the individual patient response and by the type of infection. The antibody response is greatest in amebic liver abscesses, less in intestinal amebiasis and still less in asymptomatic cyst passers. Thus, serological testing for serum antibodies to E. histolytica has been shown to detect 90 - 100% of amebic liver abscesses, 80 - 100% of symptomatic intestinal amebiasis and 10 - 50% of asymptomatic cyst passers.

Principle of the test

On the surface of the microtiter wells, specific antigens of Entamoeba histolytica are bound. Diluted serum samples and controls are pipetted into the wells and incubated at room temperature. Present antibodies bind to the immobilized antigens. Unbound material is removed in a washing step.

In a second step, a POD-conjugated Protein A is added; which has a high affinity to the Fc unit of human IgG. After incubation, unbound conjugate is removed by washing. Substrate (urea peroxide) and chromogen (TMB) are added to the wells and incubated at room temperature. The enzyme bound in the wells converts the colorless substrate/chromogen to a blue color. Addition of stop solution converts the color from blue to yellow. The absorption is measured at 450 nm wavelength (optional : reference wavelengt ³ 620 nm)

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results :

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

4. Discard the internal transparent stoppers, that come in the vials of some of the reagents (Sample Diluent, Wash Solution, etc. once these vials are opened for the first time. The purpose of this stopper is to avoid leakage during transport.

5 Once the assay has been started, all subsequent steps should be completed without interruption and within the recommend time limits.

6. Use a separate disposable tip for each specimen to prevent cross-contamination.

7. Do not use or mix components from different lots.

8. Do not use reagents after expiration date.

9. It is recommended to dispense in a clean test tube the amount of reagent needed for each run in order to prevent possible contamination of the vials content(specially the Substrate A and B solutions). Clean the micropipettes after each reagent dispensing step.

10 All specimens and controls should be run at the same time so that all testing conditions remain the same.

11. The quality control specimens(controls) should be run every time samples are assayed to check the test performance.

12. Check both precision and accuracy of the laboratory equipment used during the procedure (micropippetes, ELISA reader, etc.).

The Health and Safety instructions listed below must be followed :

1. Do not smoke, eat, drink or pipet by mouth in the laboratory.

2. Wear disposable gloves whenever handling patient specimens.

3. Use a 5% sodium hypochlorite solution to wipe up spills.

4. Treat all materials used in the test as if they were infectious. Autoclave all materials for 1 hour at 121,5°C.

Add sodium hypochlorite to liquid waste and disposable material in order to reach a final concentration of 5%. Leave the material at least 30 minutes in this solution. Incinerate all disposable material.

5. All serum samples must be considered as potential infectious and must be handled with usual precautions.

6. The positive and negative controls have been tested and found nonreactive to HBsAg, HIV and other infectious agents. As no available test methods can offer, presently, complete assurance that these agents are absent from the sample tested, all the controls must be regarded as potentially infectious and must be handled with usual precautions.

7. Avoid contact of substrate and stop reaction solutions with skin and mucosa (possible irritation, burn or toxicity hazardous). In case of contact, rinse the affected zone with plenty of water.

8. Handling and disposal of chemical products must be done according to good laboratory practices(GLP).

Material provided

The reagents in one package are sufficient for 96 determinations.

Each test kit contains :

1 x 12 Microwell Strips (breakable) with 8 wells
each in a frame; coated with inactivated Entamoeba histolytica antigen;

in a resealable foil bag.

2 x Sample Buffer (50 ml, pH 7.4), ready to use, dyed yellow, colorless lid

1 x Washing Buffer (50 ml, ph 7.4), 20x conc., dyed blue, brown lid

1 x Positive Control (1.2 ml), red lid;
inactivated human serum, ready to use

1 x Negative Control (1.2 ml), colorless lid;
inactivated human serum, ready to use

1 x Protein A Conjugate (6 ml), red lid;
HRP-Conjugated Protein A, ready to use

1 x Substrate (6 ml), green lid;
urea peroxide, ready to use

1 x Chromogen (6 ml), blue lid;
tetramethylbenzidine (TMB), ready to use

1 x Stop Solution (6 ml), yellow lid;
1 M sulfuric acid

1 x Insert

Material needed but NOT provided

- Distilled or deionized water

- Vortex mixer

- Micropipets for volumes of 10 - 100 µl and 100 - 1000 µl

- Measuring cylinder (1000 ml)

- Microplate washer or multichannel pipet

- Microplate reader (450 nm,)

- Absorbent paper

Stability and storage

Reagents must be stored at temperatures between 2°C and 8°C.

Expiration date is the last day of the month of the expiration date found on the box, vials and microstrips labels.

Exposure of the reagents to ambient temperatures for short periods does not affect the stability of the reagents.

DO NOT FREEZE ANY OF THE REAGENTS. Freezing can cause irreversible damage to the reagents.

The colorless chromogen must be protected from exposure to direct light to avoid deterioration or coloration by autoxidation. If the chromogen has turned blue, the reagent shoud be discarded.

The following criteria may indicate a reagent deterioration :

- a turbidity or a blue coloration of the colorless chromogen prior to its use

- an absorbance value (O.D.) of the negative control at 450 nm > 0.3

- an absorbance value (O.D.) of the positeve control at 450 nm < 0.8

Serum Specimens Information

Fresh Serum or Plasma are required. Serum may be stored at 2°-8°C for op to seven (7) days with 0,02 % thimerosal. If longer storage is desired, store at -20°C for up to one(1) year (avoid repeat freezing and thawing of samples, it may cause false results). DO NOT USE SODIUM AZIDE, this product may inhibit the peroxidase enzymatic reaction.

Allow the sample to stand till the blood has coagulated. Centrifugation can be used to separate the serum from the rest of the components.

Serum samples demonstrating gross lipemia, gross hemolysis, turbidity or microbial contamination should not be used with this test.

Diluted samples can be used for up 7 hours if they have been stored at 2 - 8 ° C.

Test Procedure

Preliminary comments

Bring all reagents and the microwell strips for at least 30 minutes to room temperature before use. Mix the reagents well before use. Reproducibility in any EIA depends on exact pipetting, the observance of incubation times and temperature and the consistency of wash sequences.

During the washing steps, take care that all wells are filled with buffer and that the liquid is completely removed from the wells. Do not allow microwells to dry between steps.

Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.

Except the washing buffer, all reagents are ready to use

Preparation of the Washing Buffer

1 part of the concentrated washing buffer is diulted with 19 parts of distilled water. Crystals in the buffer concentrate can be dissolved in a waterbath at 37 ° C.

Add 50 ml of the concentrated washing buffer to a 1000 ml graduated cylinder.

Bring the final volume to 1000 ml with distilled or deionized water. The diluted washing buffer can be used up to the expiry date printed on the labels if stored at 2 - 8 ° C.

Preparation of the samples

Before starting the test, serum samples have to be diluted 1:100 with the sample buffer.

e.g. 10 µl serum + 990 µl sample buffer

Attention !

The controls included in the kit are ready to use and must not be diluted.

First incubation

After insertion of a sufficient number of wellstrips into the microwell holder, 50 µl of the positive control, the negative control and the diluted sera are pipetted into the wells and incubated for 15 min at room temperature. It is recommended to include two negative controls in each run.

Washing

Decant or aspirate all microwells into a waste container with a disinfectant. Ensure complete removal of the liquid from the microwells by tapping the inverted plate onto absorbent paper.

Then wash all wells 5 times with 300 µl of prepared washing buffer. Be sure to remove residual washing solution by firmly tapping the inverted microwells on absorbent paper after single washing steps.

If a microplate washer is used, take care that the washer is adjusted to the used microplate type.

Second incubation

Add 50 µl or 1 drop of protein A conjugate to all wells. Incubate the plate for 15 min at room temperature.

Washing

Wash 5 times according to the previous washing step.

Third incubation

Add 50 µl or 1 drop of substrate and 50 µl or 1 drop of chromogen into each well.

Incubate the plate for 15 min at room temperature.

Following the incubation, the reaction is stopped by adding 50 µl or 1 drop of stop solution to each well. After careful mixing (soft tapping on the edge of the plate) the absorbance is measured in a microplate reader at 450 nm .

Remark :

Highly positive patient samples can cause dark precipitates of the chromogen.

Quality control

For the quality control, positive control and negative control (double determination) must be carried along with each test procedure. The test was carried out correctly if the positive control shows an absorbance value (O.D.) at 450 nm greater than 0.8 The mean absorbance value of the negative control at 450 nm must show a value lower than 0.3. If the single measurements diverge from the mean absorbance value more than 25 %, the test must be repeated.

Calculation of the sample ratio:

a) Calculate the average O.D. of the negative control.

b) Add 0.150 to the average O.D. The result is the cut-off value of the assay.

c) Calculate the sample ration by dividing the sample O.D. throught the cut-off value obtained in point b).

E.g. negative control well 1 O.D.=0.115
negative control well 2 O.D.=0.125
sample O.D.=0.508

$$\text{cut-off value} = \frac{0.115 + 0.125}{2} + 0.150 = 0.270$$

$$\text{sample ratio} = \frac{0.508}{0.270} = 1.88$$

Valuation of sample ratio

	negative	equivocal	positive
Sample ratio	< 0.9	0.9-1.1	>1.1

Remarks about the test procedure and interpretation

The E. histolytica (Ab) EIA kit detects specifically antibodies against Entamoeba histolytica. It should be used in suspected cases of amebiasis. Assay results should always be interpreted in connection to the clinacal diagnosis and other diagnostic results.

With amoeba-positive findings in stool, a positive antibody detection enables identification of E. histolytica sensu stricto as cause for the clinical symptoms.

In the case of extraintestinal complications a positive antibody detection can indicate an amebiasis, even though stool findings are negative.

The antibody findings should be confirmed with clinical symptoms and other diagnostic methods.

Negative antibody findings cannot exclude an Entamoeba infection. Due to low antibody titer at very early time of an infection, the test can show negative or equivocal results. If a clinical suspicion subisists, after a few days another patient's sample should be tested.

A positve result does not exclude the presence of other pathogens.

References

1. Healy G., Bull N.Y. Acad. Med., 47, 478 (1971).

2. Healy G., Rev. Infect. Diseases, 8 (2), 239 (1986).

3. Healy G. et al., Arch. invest. Med., 5, 495 (1974).

4. Patterson, M. et al., Gastroenterology, 78, 136 (1980).

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QUICK REFERENCE TEST PROCEDURE

1. Dilute washing solution.

2. Dilute the samples 1 : 100 using the sample buffer.

3. Add 50 µl of diluted samples or controls to each well. Gently shake for 15 seconds.

4. Incubate 15 minutes at Room Temperature (20-25°C)

5. Throw the well content and wash 5 times with 300 µl of washing buffer.

6. Add 50 µl of protein A conjugate to each well.

7. Incubate 15 minutes at Room Temperature (20- 25°C).

8. Throw the well content and wash 5 times with 300 µl of washing buffer.

9. Add 50 µl of Substrate to each well. Add 50 µl of chromogen to each well.

10. Incubate 15 minutes at Room Temperature (20- 25°C) in the dark.

11. Add 50 µl of Stop Solution to each well.

12. Read Optical Densities with an ELISA reader with a 450 nm filter.